IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Application of: Moore et al.

Application Number: 09/263,626

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Title: Cytokine Receptor Common Gamma Chain Like

Group Art Unit: 1646

Examiner: Brannock, M.

Attny. Docket No.: **PF466**

DECLARATION OF PAUL A. MOORE UNDER 37 C. F. R. § 1.132

Assistant Commissioner for Patents
Washington, D.C. 20231

Sir:

I, Paul A. Moore, a citizen of the United Kingdom, do hereby declare and state:

1. I am a named inventor of the captioned application, which is assigned to Human Genome Sciences, Inc. (HGS). From March 1993 to August 1995 and from July 1996 until present, I have been employed by HGS, where I currently hold the position of Associate Director of Pre-Clinical Discovery. The work described below was done either by myself or under the supervision of either myself or the other named inventors of the application, Steven M. Ruben or Craig A. Rosen.

2. The present invention provides isolated polynucleotides encoding a Cytokine Receptor Common Gamma Chain Like (CRCGCL) protein having the amino acid sequence shown in Figure 1A-B (SEQ ID NO:2) of the captioned application (*see, e.g.,* Figure 1A-B attached as Exhibit A). The CRCGCL protein of the present invention shares sequence homology with the IL-2 receptor common gamma chain. Figure 2 of the specification is an alignment of CRCGCL and the IL-2R common gamma chain of Bos Taurus illustrating the sequence homology between them (*see, e.g.,* Figure 2 attached as Exhibit B).

3. Experiments under my supervision, or under the supervision of the other inventors listed in paragraph 1 of this document, have been performed which indicate that CRCGCL activates the Jak-STAT signal transduction pathway and binds a cytokine.

4. A 293T reconstitution cell assay was used to assess whether CRCGCL polypeptides activate the Jak-STAT pathway (as measured by tyrosine phosphorylation), as has been shown for the IL-2R common gamma chain. 293T cells were transfected by the Ca_2PO_4 method, using 2μg of each construct, never to exceed 10 μg per plate, as shown in the upper panel of the figure in Exhibit C. As a control, pCDNA3 was added as necessary to equalize the amount of DNA being transfected in each experiment. The medium was changed after 16 hours, and cells were cultured in complete DMEM for an additional 24 hours. Cells were then stimulated with the supernatant from cells expressing a cytokine of interest (either IL-7, IL-2, Thymic Stromal Lymphoprotein (TSLP)) or a no cytokine control (the vector alone, pC4), washed in PBS, and lysed in Brij lysis buffer (10mM Tris, pH 7.5, 0.875% Brij, 0.125% NP40, 2.0mM EDTA, 150mM NaCl). 300-500μg of total protein was immunoprecipitated (IP) with the appropriate Jak or STAT family kinase antibody of interest (*e.g.*, STAT5 or Jak 2, Santa Cruz Biotechnology, Inc. Santa Cruz, CA), separated on 8% SDS-PAGE gels and transferred to Immobilon-P membranes. The membranes were blotted with anti-phosphotyrosine antibody 4G10 (UBI), and detected using chemiluminescence. Increased phosphorylation of STAT5 and Tyk2 was detected using this assay when CRCGCL was cotransfected with IL-7R alpha chain, Jak2, and STAT5 and stimulated with a cytokine, TSLP (*see, e.g.*, the western blot shown in the lower panel of the figure provided as Exhibit C). Some stimulation was seen in the transfected cells in the absence of cytokine (e.g., supernatants from cells expressing vector alone, pC4 lanes in Exhibit C). This is a common result in reconstitution systems due to the fact that the kinase molecules autoactivate because of overexpression. However, the interaction between the cytokine, TSLP, and CRCGCL, and the activation of the Jak-STAT signal transduction pathway was shown to be specific by the fact that the increased STAT5 and Tyk2 phosphorylation could be inhibited by addition of a soluble extracellular domain of CRCGCL fused to Fc (Fc) (e.g., for 293T cells transfected with

CRCGCL, IL7Ralpha, STAT5, Jak2 and Tyk2, compare the western blot lanes labeled TSLP to those labeled TSLP+Fc). The results of these experiments indicate that (1) CRCGCL binds a cytokine and activates the Jak-STAT signal transduction pathway and (2) that the soluble extracellular domain of CRCGCL binds a cytokine and inhibits the activity of CRCGCL in activating the Jak-STAT pathway.

5. In addition, flow cytometry was used to measure whether CRCGCL polypeptides bind a cytokine. A shift in the mean fluorescent intensity measured by FACScan on cells alone compared to cells treated with a ligand suggests that the ligand has associated with a cell surface molecule. FACScan analyses were performed on 293T cells which had been transfected with either CRCGCL, IL-7R alpha chain (IL7a), or IL-2R common gamma chain (IL2 common gamma C) alone, or in combination with one another, treated with a FLAG-tagged TSLP molecule bound to an anti-FLAG biotin conjugate and fluorescein isothiocyanate labeled streptavadin. A shift in the mean fluorescent intensity as measured by FACScan was detected when 293T cells transfected with CRCGCL alone and in combination with IL-7R alpha chain were treated with the FLAG-tagged TSLP. The results of these experiments which are shown in the attached graphs (*see, e.g.,* Exhibit D) indicate that CRCGCL binds a cytokine.

6. These experimental results demonstrate that a CRCGCL polypeptide: (1) binds a cytokine; and (2) activates the Jak-STAT signal transduction pathway.

7. The fact that CRCGCL has immune specific expression (*see, e.g.,* the captioned application at page 7, lines 15-18), binds a cytokine, and activates the Jak-STAT signal transduction pathway, indicates that CRCGCL is involved in the modulation of immune cell proliferation and/or differentiation.

8. I further declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true and further that these statements are made with the knowledge that willful false statements and the like are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United

States Code, and that such willful false statements may jeopardize the validity of the application or any patents issued thereupon.

2/27/01
Date

Paul A. Moore
Paul A. Moore, Ph.D.